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INACTIVATION OF HEPATITIS A VIRUS (HAV) BY CHLORINE AND IODINE IN WATER

ANNUAL REPORT

by

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November, 1986

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5102

Contract No. DAMD17-86-C-6053

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SUMMARY

Studies were done in a batch reaction system to determine the kinetics and extent of inactivation of aggregated preparations of hepatitis A virus (HAV), strain HM175, poliovirus type 1, strain LSc, and echovirus type 1, strain V239, by 1 mg/l free chlorine and one tablet per quart Army iodine in halogen demand-free water buffered at pH 4.5, 7.0 and 9.5 and at temperatures of 5 and 25°C. The results obtained so far indicate that HAV is rapidly inactivated by 1 mg/l free chlorine under these conditions, with times for 99.99% inactivation (T99.99) of <10 minutes at 5°C and <15 minutes at 25°C. Polio 1 and echo 1 also were inactivated rapidly under most of these same conditions. The exception was pH 9.5, where times for 99.99% inactivation of polio 1 and echo 1 were 65 and 147 minutes, respectively, at 5°C.

In experiments done so far, HAV was inactivated rapidly by one tablet per quart iodine in buffered, halogen demand-free water at pH 7.0 and 9.5, with T99.99 values of <1 minute. In contrast, polio 1 and echo 1 were not inactivated rapidly by this concentration of iodine at pH 4.5 and 7.0.

The results obtained so far indicate that the HM175 strain of HAV is inactivated rapidly by free chlorine and Army iodine. However, further studies must be done in order to determine the sensitivity of HAV to these halogens in the presence of appreciable halogen demand and other potential interferences. In addition, studies must be done with other strains of HAV in order to determine if their sensitivity to chlorine and iodine is similar to or different from that of the HM175 strain of HAV.

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I. INTRODUCTION AND BACKGROUND

A. Importance and Epidemiology of Hepatitis A Virus in Water

Hepatitis A or infectious hepatitis is a common and epidemiologically important waterborne viral disease. Outbreaks due to the transmission of hepatitis A virus (HAV) by the water route have most often been attributed to fecal contamination of untreated or inadequately treated water (Lippy and Waltrip, 1984). The risks of hepatitis A infection and illness from drinking untreated, undisinfected water are demonstrated by a recent waterborne outbreak of hepatitis A in a small, rural community in western Maryland (Sobsey et al., 1984). Fourteen cases of hepatitis A occurred in this community of 300 people who were drinking untreated groundwater from household wells. Groundwater was fecally contaminated, probably by septic tank effluent, and contained high levels of total and fecal coliform bacteria. HAV was detected and quantified in four of six concentrate samples of incriminated groundwater by inoculation of African green monkey kidney cell cultures and by experimental infection of chimpanzees. This report demonstrates the risks of hepatitis A from consumption of untreated, fecally contaminated water, and it underscores the need to disinfect individual water supplies under field conditions with a disinfectant that is effective for HAV.

A recent waterborne outbreak of viral gastroenteritis and hepatitis A in Georgetown, Texas, highlights the potential for transmission of HAV in communities relying on limited water treatment practices involving primarily disinfection (Hejkal et al., 1982). Hepatitis A antigen was detected by radioimmunoassay in the contaminated water, and viral disease transmission occurred even though chlorinated groundwater samples taken from the distribution system were negative for coliform bacteria. The occurrence of 36 reported cases of hepatitis during the outbreak demonstrated the failure of conventional indicator bacteria to adequately predict viral contamination of the water supply, as well as the ability of HAV to survive the chlorination process.

Other recent reports also suggest that HAV may be more resistant to various chemical and physical agents (Siegl et al., 1984) and more stable under various environmental conditions (Sobsey et al., 1986) than other viruses and bacteria.

Conventional water treatment practices utilizing chemical disinfection, primarily chlorination, are generally believed to be effective in producing microbiologically safe drinking water.

However, the growing number of reports on the isolation of viruses from treated drinking water (Bitton et al., 1986) suggest that viruses may survive treatment under certain conditions. The establishment of reliable water treatment practices and water quality standards to insure the virological safety of water supplies can be achieved only by fully understanding the response of HAV to water disinfectants such as chlorine and iodine.

B. Previous Studies on Disinfection of HAV in Water.

Despite the need to determine the kinetics and extent of HAV inactivation by water disinfectants, the few investigations reported to date on HAV inactivation by chlorine have been inadequate due to technical limitations. Early studies by Neefe et al. (1945, 1947) provided indirect evidence that HAV is insensitive to combined chlorine. Using human volunteers for virus infectivity assay, Neefe and co-workers found that a total chlorine residual of 1 mg/l did not completely inactivate HAV in dilute fecal suspensions after a contact time of 30 minutes. The addition of sufficient chlorine to produce total and free chlorine concentrations of 1.1 and 0.4 mg/l, respectively, in purified effluent was required to prevent clinical manifestations of infectious hepatitis in the volunteers. More recently, Peterson et al. (1983) used marmosets to assay for HAV infectivity after chlorination of a partially purified preparation of HAV. The infectivity of the preparation, which contained about 1500 infectious units/ml, was only partially reduced by treatment with up to 1.5 mg/l of free residual chlorine at neutral pH for 30 minutes. These results, along with observations made during the outbreak of hepatitis in Georgetown, Texas (Hejkal et al., 1982), suggest that HAV is more resistant to conventional water chlorination processes than other enteroviruses and indicator bacteria. In contrast, results of studies by Grabow et al. (1983) indicated that HAV may be more sensitive to free chlorine than previous studies and epidemiological evidence have suggested. Using serological techniques for assay of HAV infectivity in cell culture, Grabow and co-workers found that HAV was very sensitive to low levels of free chlorine relative to selected indicator viruses and bacteria. However, other studies by this group indicated that HAV was relatively resistant to combined forms of chlorine (Grabow et al., 1984).

C. Military Need for Virus Disinfection of Water.

In the military there is a special need for disinfectants that will be effective in destroying waterborne pathogens under adverse or emergency conditions, particularly when the quality

of the water available for consumption is poor. Since World War II, the Armed Forces of this country have relied primarily on globaline tablets, an iodine-based disinfectant, for disinfection of canteen water and other small-volume field water supplies (O'Conner and Kapoor, 1970). Relatively little is known about the adequacy of this disinfectant in preventing the transmission of viral pathogens such as HAV in waters with varied physical and chemical characteristics. Outbreaks of infectious hepatitis associated with military activities have continued to occur since the development of globaline (Bancroft and Lemon, 1984). Although the effectiveness of globaline and other forms of iodine against HAV has not been evaluated, disinfection studies on their effectiveness for other enteric viruses, enteric bacteria and protozoan cysts have been reported (Safe Drinking Water Committee, 1980). However, considering the substantial differences in the response of different enteric viruses to chlorine (Liu et al., 1971), it is impossible to predict the effectiveness of globaline or other disinfectants for inactivation of HAV in water.

In view of the limited data on HAV disinfection in general and the inconsistent findings of the few studies on HAV disinfection by chlorine, a critical evaluation of HAV inactivation by free and combined forms of chlorine and by iodine (globaline) is clearly warranted. Water quality variables, such as the presence of suspended inorganic particulates and soluble and colloidal organic matter, are important factors that need to be evaluated for their effect on the efficiency of disinfection of HAV. The adsorption of viruses to particulate matter in water has been well documented (Goyal and Gerba, 1979; Hurst et al., 1980; Schaub and Sagik, 1975; Sobsey et al., 1980). Particulates may protect viruses in the aqueous environment by sheltering viruses from disinfectant exposure or by consuming or chemically changing the disinfectant. Naturally-occurring soluble and colloidal organic matter (such as humic and fulvic acids) in natural waters, finished waters and wastewaters may also be a factor in reducing the efficacy of the disinfection process by consuming or changing the active species of the chemical agent. The effects of suspended matter and soluble and colloidal organic matter on HAV disinfection by chlorine and iodine have not been adequately addressed in the recent studies reported in the literature.

The study of HAV inactivation kinetics by chlorine and iodine is now feasible with the utilization of new methodologies for the cultivation and enumeration of HAV in cell cultures (Daemer et al., 1981; Frosner et al., 1979; Lemon et al., 1983; Provost and Hilleman, 1979). The focus of this proposed project is to examine the kinetics and extent of HAV inactivation by chlorine

and iodine, with special emphasis on determining the influence of important water quality variables on chlorine and iodine inactivation of HAV and, for comparison, selected model viruses.

II. STATEMENT OF THE PROBLEM AND OBJECTIVES

A. Statement of the Problem

The problem to be studied is the efficiency (kinetics and extent) of disinfection (inactivation) of hepatitis A virus by chlorine (calcium hypochlorite) and Army iodine (globaline) in waters of different quality. In some experiments, the inactivation of other viruses or of different strains of HAV will be compared, and in other experiments the inactivation of HAV will be compared to the inactivation of Escherichia coli.

B. Specific Ojectives

The specific objectives of this study are to determine the kinetics and extent of HAV inactivation by free (hypochlorite ion/hypochlorous acid) and combined (monochloramine) forms of chlorine and by iodine (globaline tablets) in waters of different quality. As an alternative to using highly purified, monodispersed virus preparations, disinfection experiments will utilize partially purified, aggregated preparations in order to better model the physical state of the viruses in natural aquatic environments.

The water quality variables to be studied are water source (buffered halogen demand-free water and untreated groundwater), particulates (in the form of bentonite clay at a turbidity of 5 NTU), soluble and colloidal organics (in the form of 1:1 mixtures of humic and fulvic acids at a concentration of 10 mg/l), pH level (pH 4.5, 7.0 and 9.5) and temperature (5 and 25°C). Virus inactivation kinetics will be determined in model waters having a range of physical and chemical qualities, and will include studies in demand-free, buffered systems and in water of poor quality (worst case). Worst case water is groundwater at pH 9.5 and 5°C that contains 10 mg/l humic and fulvic acids (1:1 mixture) and 5 NTU bentonite clay turbidity.

Waters are seeded with a mixed virus preparation containing sufficient quantities of HAV, poliovirus 1, echovirus 1 and MS2 bacteriophage to follow the inactivation of each virus over at least 4 $\log_{10}$ units (99.99%). The latter three viruses are included in order to compare HAV sensitivity to halogen disinfectants to that of other viruses which have been previously studied in this regard.

III. METHODS AND MATERIALS

A. Viruses, Cell Cultures and Virus Purification

1. HAV. The HM175 (NIH prototype) strain of HAV, originally isolated from feces of an infected human in Australia (Daemer et al., 1981; Lemon et al., 1983; Gust et al., 1985) was produced in persistently infected BS-C-1 cells grown in 850 cm^2 roller bottles or 6000 cm^2, ten-tiered cell factories (NUNC) incubated at 37°C. Prior to persistent infection, the virus had been serially passaged 6 times in marmosets, 10 times in primary African green monkey kidney (AGMK) cells and 7 times in BS-C-1 cells.

HAV infectivity was assayed by the radioimmunofocus assay (RIFA) in BS-C-1 cells as previously described (Lemon et al., 1983; Sobsey et al., 1985), except the incubation period was reduced to one week. The RIFA is an enumerative assay analogous to a plaque assay, except that non-cytopathic, focal areas of infected cells are visualized by an immune autoradiographic method.

Persistently infected cells were passaged every two to four weeks by trypsinization and then resuspension of some of the cells in growth medium at a concentration of about 1×10^5 cells/ml for re-inoculation into culture vessels. At each passage, some of the persistently infected cells and all of the culture fluids were harvested as crude virus stock. Harvested infected cells were centrifuged at low speed (about 3000 x g), resuspended in small volumes of phosphate-buffered saline (PBS), pH 7.5, and extracted with an equal volume of chloroform. The HAV-containing PBS was recovered by low speed centrifugation to remove cell debris and chloroform. The cell debris and chloroform was extracted four to six more times with equal volumes of PBS to obtain additional virus, and all PBS extracts were pooled as virus stock. HAV in culture fluids was concentrated by precipitation with polyethylene glycol (PEG) 6000 (12% w/v, pH 7.2) overnight at 4°C. Resulting precipitates were recovered by low speed centrifugation, resuspended in a small volume of PBS and extracted with a volume of chloroform equal to the PBS volume in order to remove excess PEG. The PBS extracts were cleared of chloroform and PEG by low speed centrifugation.

PBS extracts of cells and PEG concentrates from culture fluids were pooled, and HAV was pelleted by ultracentrifugation at 30,000 RPM (105,000 x g) for 4 hours at 5°C. Resulting pellets were resuspended in small volumes of 0.05M phosphate-buffered distilled water (PBDW) and supplemented with CsCl to give a

density of 1.33 g/ml. These samples were ultracentrifuged to equilibrium in self-generated gradients at 25,000 RPM (90,000 x g) and 5°C for 3 days using the SW27 rotor ((Beckman Instruments). Gradients were harvested in fractions from the bottoms of the tubes and assayed for HAV infectivity by RIFA. Fractions with the peak of HAV infectivity were desalted by untrafiltration and washing with PBDW using Centricon 30 tubes (Amicon Inc). Desalted fractions were layered onto 10-30% sucrose gradients in phosphate buffered halogen demand-free water, pH 7.5, (PBHDFW) and ultracentrifuged in the SW27 rotor at 25,000 RPM (90,000 x g) and 5°C for 5.5 hours. Under these conditions, single virions should sediment about 2/3rds of the distance from the top to the bottom of the gradient. Gradient fractions were harvested from the top of the tube and assayed for HAV infectivity by RIFA. Gradient fractions were characterized as containing single virions or small, medium or large aggregates of HAV according to their position in the gradient. HAV gradient fractions were then pooled and mixed with appropriate amounts of gradient fractions of the other test viruses such that the total amount of each virus consisted of about 8% single virions, 19% small aggregates, 39% medium aggregates and 34% large aggregates. The numbers of virions in aggregates of fractions having small, medium and large aggregates has not yet been determined. The titer of each virus in the mixture was 1-5 x 10^8 infectious units/ml. Virus mixtures were further diluted 1:5 in halogen demand-free water for use in disinfection experiments in order to reduce halogen demand.

2. Echovirus 1 and Poliovirus 1. Echovirus 1 (strain V239) and poliovirus 1 (strain LSc) were grown and assayed by the plaque technique in BGM (African green monkey kidney-derived) and MA104 (rhesus monkey kidney-derived) continuous cell lines, respectively, as previously described (Sobsey et al, 1978). In order to assay each animal virus type (HAV, poliovirus and echovirus) in samples containing all three viruses, the other two viruses were neutralized by adding antibodies (antisera) against them to the virus diluent. For example, poliovirus was assayed by neutralizing echovirus type 1 using antiserum against echovirus type 1 in the poliovirus diluent. HAV did not have to be neutralized in assays for poliovirus or echovirus because it was non-cytopathic, grew slowly and did not interfere with the assays for these other two viruses (unpublished results).

Poliovirus and echovirus were first plaque-purified 2-3 times and then grown in large quantities under either one-step growth conditions (>5 PFU/cell) or at low multiplicity of infection (MOI; 0.01-0.1 PFU per cell). Crude virus stocks were harvested

from infected cells at 5-7 hours post-infection under one-step growth conditions or from infected cell lysates several days post-infection at low MOI when cytopathic effects were 4+. Virus was liberated from cells and cell debris by freezing and thawing, and then cell debris was removed by centrifugation at low speed (10,000 x g for 15-30 minutes). Viruses in resulting supernatants were pelleted by ultracentrifugation (105,000 x g and 5°C for 4 hours). Resulting virus pellets were resuspended in buffered HDFW, homogenized 1 minute, and in some cases centrifuged at 10,000 x g and 5°C for 20 minutes to remove additional debris. After supplementing the sample with CsCl to give a density of 1.33 g/ml, viruses were banded to equilibrium as for HAV. Gradient fractions were harvested and assayed for virus infectivity, and virus peak fractions were desalted using Centricon 30 ultrafiltration units. These fractions were pooled and subjected to rate-zonal centrifugation in 5% (or 10%) to 30% sucrose gradients as for HAV. Gradient fractions were harvested and assayed for virus infectivity and appropriate amounts of virus fractions were added to HAV samples to give the desired distribution and virus titers of single virions as well as small, medium and large aggregates.

3. Bacteriophages. Bacteriophages MS2 (ATCC 15597-B1) and ϕX174 (ATCC 13706-B1) were grown and assayed by the top agar plaque technique (Adams, 1959) in E. coli C3000 (ATCC 15597) and E. coli C (ATCC 13706) hosts, respectively, using nutrient agar #2 (nutrient agar with 0.5% NaCl) medium. Crude virus was harvested from the top agar of plaque assay plates having confluent lysis by scraping into small volumes (3-5 ml/plate) of PBS. Harvests were frozen and thawed, homogenized or vortex mixed 1-2 minutes, and then centrifuged at 10,000 x g for 15-20 minutes to remove cell debris and agar. Viruses in the resulting supernatant were pelleted by ultracentrifugation for 4 hours at 105,000 x g and 5°C. Pellets were resuspended in PBHDFW, supplemented with CsCl to give a density of 1.44-1.45 g/ml and the viruses were banded to equilibrium in self-generating CsCl gradients for 3 days at 25,000 RPM and 5°C using the SW27 rotor. Gradient fractions were assayed for virus infectivity, and virus peak fractions were desalted using Centricon 30 ultrafilters. Desalted fractions were layered onto 10-30% sucrose gradients in PBHDFW and centrifuged at 36,000 RPM and 5°C for 4.5 hours (MS2) in the SW41 rotor (Beckman Instruments) or 25,000 RPM and 5°C for 10 hours (MS2) or 7 hours (ϕX174) in the SW27 rotor. Gradient fractions were collected from the top of the tubes and assayed for virus infectivity to determine the location of virus peaks.

It was found that both MS2 and ϕX174 from sucrose gradients were unstable after sucrose exposure and rapidly lost infectivity

titer when stored at 4°C or other storage temperatures. Attempts to preserve virus infectivity by dilution of the sucrose gradient fractions or by other means were unsuccessful. Subsequent experiments in which these phages were simply placed in sucrose solutions confirmed that exposure to sucrose caused virus instability. For this reason, MS2 or other bacteriophages could not be included in disinfection studies. Although virus stocks could be prepared without using sucrose density gradients, such stocks could not be prepared by a method that would give specific proportions of viruses aggregated to different extents. Therefore, the bacteriophage stocks could not be prepared with a known and controlled amount of virus aggregation as was done for the animal viruses and could not be evaluated under the same protocol.

B. Glassware and Halogen Reagents.

All glassware for disinfection experiments and preparation of halogen demand-free (HDF) virus stocks was soaked at least 4 hours in a strong chlorine (10-50 mg/l) solution and then rinsed thoroughly with halogen demand-free water (HDFW) prior to use. HDFW and buffer solutions for disinfection experiments were prepared from glass-distilled, deionized water by adding chlorine to approximately 10 mg/l. After storage at room temperature for at least 1/2 day, water or buffers were dechlorinated by exposure to a submersible ultraviolet light. HDF, phosphate-based buffers, 0.01M, were used to prepare chlorine test solutions and buffered water for disinfection experiments.

Reagent grade calcium hypochlorite was used to prepare solutions of hypochlorous acid (HOCl) at pH 4.5, hypochlorite ion (OCl^-) at pH 9.5, and mixtures of these free chlorine species at pH 7.0. Hypochlorite stock solutions of about 100 mg/l were prepared by dissolving about 0.2 g of $Ca(OCl)_2$ in 1 liter of HDFW. Stock solution was then diluted in test water (halogen demand-free, 0.01M phosphate buffer, pH 4.5, 7.0 or 9.5 in initial experiments) to give the target chlorine concentration. Target chlorine concentration was verified by chemical analysis. Iodine solutions were prepared from globaline tablets which contain tetraglycine hydroperiodide as the active ingredient and disodium dihydrogen pyrophosphate as a buffer. Globaline concentrations to be tested are the molar equivalent of 1 mg/l chlorine (3.6 mg/l iodine) as well as two concentrations based upon recommended Army field use (1 to 2 tablets per quart, giving concentrations of about 8 and 16 mg of titratable iodine per liter, respectively). Iodine solutions were prepared by dissolving 1 or 2 globaline tablets in about 900 ml of HDFW and adjusting to pH 4.5, 7.0 or 9.5 with NaOH or H_2SO_4. For

experiments testing disinfection efficiency of one or two tablets per quart, these samples were brought to a volume of 927 ml with HDFW and iodine concentration was measured. The volume of iodine solution was such that addition of 1 part of test virus mixture to 9 parts of iodine solution would dilute the iodine to the target concentration. In experiments, samples with 1 or 2 tablets per quart had to have initial measured iodine concentrations of >7.5 and >15 mg/l, respectively. For iodine solutions that were to be the molar equivalent of 1 mg/l chlorine, the iodine concentration of a solution containing 1 tablet per quart was adjusted to 3.6 mg/l by dilution with HDFW. Overall, the iodine concentrations obtained in this study were generally consistent with those of previous studies. For example, Farrah (1986) used one or two globaline tablets per quart in studies on the inactivation of three enteroviruses and bacteriophage MS2. At one tablet per quart, his initial iodine concentrations at pH 5.0 ranged from 6.7 to 7.2 mg/l, and after 60 minutes they ranged from 7.0-7.1 mg/l. In this present study initial iodine residuals ranged from 8.8 to 9.0 mg/l, and after 60 minutes they were 7.7 to 3.9 mg/l. The greatest iodine losses were at pH 9.5 where HOI, the hydrolysis product of I_2, decomposes to iodate and iodide, as previously observed (Chang, 1958). Although the Army specifications and test protocol for iodine tablets indicate somewhat higher iodine concentrations than reported here, there are differences in test protocols and analytical methods. Furthermore, this study was done using a single lot of Army approved globaline tablets. These tablets could have been somewhat deficient in potency or they could have lost potency during continued storage and use. Since the tablets had not reached their expiration or re-test date, they were considered representative of tablets in field use.

C. Halogen Analysis.

Iodine and chlorine concentrations were measured by DPD colorimetric methods as described in Standard Methods for the Examination of Water and Wastewater, 16th edition (American Public Health Association, 1985). Standardization of procedures for chlorine measurement was by the DPD ferrous titration method, and for iodine measurement by using potassium bi-iodate as a primary standard. These methods were judged to be more reliable than amperometric titration for at least two reasons. First, amperometric titration would have required substantial futher dilution of test samples due to the volume requirements of the instrument. Such dilution could lead to futher analytical errors and would have been near the lower detection limit of the amperometric titrator. Second, the amperometric titration method for iodine requires acidification of the sample to pH 4.0, which could alter the proportions of iodine species present, create or destroy other oxidants, and thereby give erroneous measurements of active iodine concentrations.

D. Protocols for Disinfection Experiments.

Initial experiments on HAV disinfection by free chlorine or Army iodine in buffered HDFW, pH 4.5, 7.0 and 9.5, were done according to the flow diagram shown in Figure 1. Samples were in 16 mm diameter x 100 mm long test tubes placed in a water bath to maintain a temperature of 5 or 25°C. For initial experiments with free chlorine at a concentration of 1 mg/l or iodine at a concentration of >7.5 mg/l (1 tablet/quart), 0.85 ml of purified virus stock mixture (HAV, polio and echo), diluted 1:5 in HDFW, was added to 7.65 ml of a chlorine solution containing 1.1 mg/l free chlorine or >7.5 mg/l iodine and briefly mixed. A second test tube containing only chlorine or iodine solution served as a halogen control. A third tube containing a 1:10 dilution of stock virus in buffered HDFW served as a virus control. Samples of 0.7 ml were withdrawn from the reaction tube (halogen solution plus added virus) for viral analysis at 0.33, 1.0, 3.0, 10, 30 and 60 minutes after virus addition. These samples were diluted two-fold immediately in virus diluent (2X Eagle's MEM) containing 1% $Na_2S_2O_3$. A further five-fold dilution (10-fold overall) followed by serial 10-fold dilutions were made in a separate diluent for each virus. These dilutions were stored at 4°C for subsequent virus assay. After the 60 minute reaction period, the remaining reaction mixture (halogen plus added virus) and the chlorine or iodine control sample (halogen only) were re-analyzed for free and combined chlorine or iodine. Samples from the virus control tube (virus plus buffered HDFW) were diluted serially 10-fold at the beginning and the end of the 60 minute reaction period for subsequent virus assay.

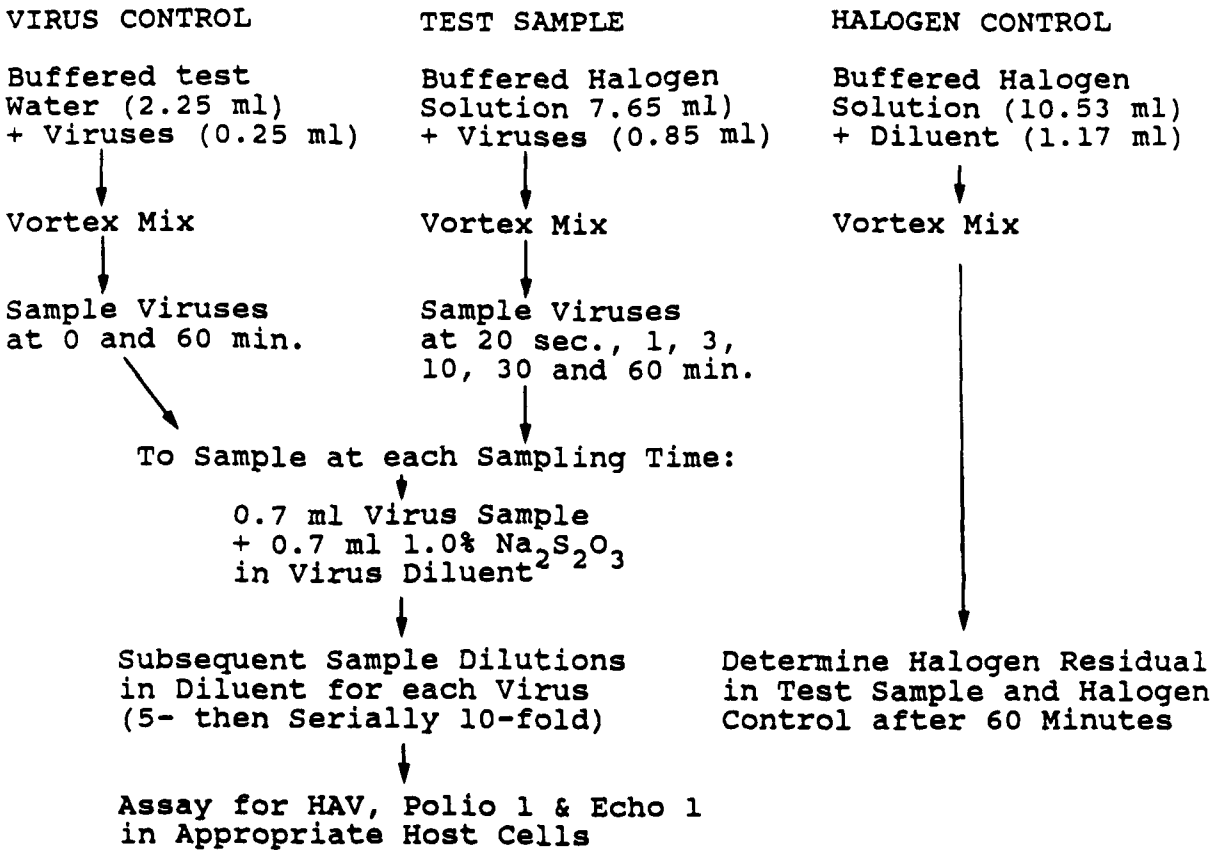

FIGURE 1. FLOW DIAGRAM OF PROTOCOL FOR HALOGEN DISINFECTION EXPERIMENTS

IV. RESULTS AND DISCUSSION

A. Disinfection by Free Chlorine in Buffered HDFW.

1. Results at 5°C. The results of initial experiments on inactivation of HAV, polio 1 and echo 1 in buffered HDFW at 5°C and pH 4.5, 7.0 and 9.5, by 1 mg/l free chlorine are summarized in Figures 2, 3 and 4 and in Table 1 as estimated times for 99.99% inactivation of the initial viruses. These are the mean results of duplicate experiments. Data for virus concentrations of samples of individual experiments are given in Appendix Tables A1-A3. Times for 99.99% inactivation of the initial virus (T99.99) were estimated from the graphs (Figures 2-4) of $\log_{10} N_t/N_o$ versus contact time, t, in minutes, where N_t = virus concentration remaining at time t and N_o = the initial virus concentration. These results show that all three viruses were inactivated rapidly by 1 mg/l free chlorine at 5°C and pH 4.5 and 7.0 and that HAV was also inactivated rapidly at pH 9.5. In all cases the T99.99 values were $\leq$12.5 minutes. In contrast, inactivation of polio 1 and echo 1 at pH 9.5 and 5°C was considerably slower, with T99.99 values of 65 and 147 minutes, respectively.

These results for polio 1 and echo 1 are generally consistent with those of previous studies in which there is greater virus inactivation at acid and neutral pH levels than at alkaline pH levels. The basis for this pH-dependent difference in free chlorine inactivation of viruses is believed to be the greater virucidal activity of HOCl, which predominates at acid pH levels, over OCl^-, which predominates at alkaline pH levels. The finding that virus inactivation rates at pH 7.0 were greater than or similar to those at pH 4.5 is difficult to explain, because the concentration of HOCl at pH 7.0 should be only about 73% of the concentration at pH 4.5, with the balance of the free chlorine at pH 7.0 (about 23%) in the form of the less virucidal OCl^-. However, other factors besides the relative amounts of HOCl versus OCl^- may be involved.

A factor that could influence the relative rates of virus inactivation at different pH levels is the stability and persistence of the free chlorine present in the reaction mixtures. In these experiments the initial concentration of free chlorine was about 1.0 mg/l after the addition of viruses. However, after 60 minutes of contact, some of the free chlorine in the reaction mixtures disappeared. Free chlorine residuals after 60 minutes of contact in reaction mixtures at pH 9.5, 7.0 and 4.5 were 0.54, 0.32 and 0.54 mg/l, respectively. These free chlorine residuals at the different pH levels do not account for the observed differences in virus inactivation rates, because

virus inactivation rates were greatest at pH 7.0, where the free chlorine residual was lowest after 60 minutes. It is important to note that at pH 4.5 and 7.0, inactivation of all three test viruses was extensive (>99.99%) within 15 minutes and when it is likely that residual chlorine concentrations were closer to the initial level of 1 mg/l.

Another factor that may influence virus inactivation rates at different pH levels is the degree of virus aggregation at each pH level. The degree of virus aggregation was controlled in the preparation of viruses, with a specific distribution of single virions and small, medium and large aggregates. However, the addition of virus stocks to reaction mixtures at pH 4.5 may have caused further aggregation of viruses, resulting in slower inactivation kinetics due to greater protection of more and/or larger aggregates. Results of previous studies have shown that acid pH levels can induce virus aggregation and decrease the rate of virus inactivation (Young and Sharp, 1985).

Yet another factor that may influence the rate of virus inactivation at different pH levels is a difference in the conformational form of the virus at each pH. One form of the virus existing at one pH may be more resistant to disinfection and/or less infectious than another form existing at another pH. Both poliovirus type 1 and echovirus type 1 can exist in at least two different, pH-dependent conformational forms (Young and Sharp, 1985). The existence of different conformational forms of HAV has not been fully established. However, preliminary evidence from this laboratory indicates the existence of possibly two conformational forms of the HM175 strain of HAV (unpublished results).

2. Results at 25°C. The results of initial experiments on inactivation of HAV, polio 1 and echo 1 in buffered HDFW at 25°C and pH 4.5 and 7.0 by 1 mg/l free chlorine are summarized in Figures 5 and 6 and Table 2 as estimated times for 99.99% inactivation of the initial viruses. Data for virus concentrations in individual experiments are given in Appendix Tables A4 and A5. The results for only one questionable experiment at pH 4.5 are reported. Results for additional experiments at pH 4.5 and 9.5 were not available for inclusion in this report due to the long time required to complete HAV assays.

The experiment at pH 4.5 was compromised because the stock virus mixture was accidentally adjusted to pH 3.8 instead of pH 4.5. Although the virus mixture was readjusted to pH 4.5 before starting the experiment, it is possible that irreversible virus aggregation occurred at pH 3.8, thereby altering the response of the viruses to free chlorine.

Another factor which must be considered in the results of the single experiment at pH 4.5 and both experiments at pH 7.0 is the loss of free chlorine from the test samples during the 60 minute reaction period. In the case of the experiment at pH 4.5 the free chlorine residual dropped to 0 at 60 minutes, while in the two experiments at pH 7.0, the free chlorine residual had dropped to 0.09 and 0.14 mg/l in the duplicate experiments. This loss of free chlorine at 25°C is not unusual at this higher temperature in a batch system held for 60 minutes. Indeed, there was an approximate loss of 50% of the initial 1 mg/l of free chlorine after 60 minutes in the chlorine control samples at 25°C. The even greater free chlorine loss in the test samples was probably due to some chlorine demand from the virus mixture. These experiments will be repeated later in the project in order to achieve target chlorine residuals.

The results of the two experiments at pH 7.0 and 25°C show that despite the considerable loss of free chlorine during the 60 minute contact period, all three viruses were rapidly inactivated, with T99.99 values of <1.2 minutes. In addition, HAV was inactivated somewhat more rapidly than polio and echo, with an estimated T99.99 of 0.4 minutes. For each virus the inactivation rate at pH 7.0 is shorter at 25°C than at 5°C, which is consistent with previous studies on virus inactivation by chemical disinfectants such as free chlorine.

As observed in the experiments at 5°C, the rates of virus inactivation at pH 7.0 are similar to or shorter than the rates at pH 4.5, although the rates for poliovirus inactivation are quite rapid at both pH levels. In contrast to the results of experiments at 5°C, HAV was more resistant to inactivation by chlorine at pH 4.5 than was poliovirus. Furthermore, the rates of HAV and echovirus inactivation at pH 4.5 were not greater at 25°C than at 5°C. As previously stated, however, the data for virus inactivation at pH 4.5 and 25°C are from only one questionable experiment in which the chlorine residual dropped to zero at some time during the 60 minute reaction period. Therefore, the experiments at pH 4.5 must be repeated in order to obtain more reliable results. Despite the problems with the experiment at pH 4.5 and 25°C, all three viruses were inactivated by >99.99% in <15 minutes.

B. Disinfection by Iodine in Buffered HDFW

The results of initial experiments on inactivation of HAV, polio 1 and echo 1 in buffered HDFW at 5°C and pH 4.5, 7.0 and 9.5 by 1 globaline tablet per quart are summarized in Figures 7, 8 and 9 and Table 3 and indicate the estimated time for 99.99% inactivation of the initial viruses. These results are for

single trials of each experimental condition tested. In some experiments there are no data for one of the three test viruses due to virus assay problems. Additional experiments have been done, but the results were not available for inclusion in this report. Data for virus concentrations in samples of individual experiments are given in Appendix Tables A6 to A8.

The results obtained so far indicate that at pH 7.0 HAV is more sensitive to inactivation by Army iodine than are polio 1 and echo 1. At pH 7.0, for example, the T99.99 for HAV is <1.0 minutes, while for polio 1 and echo 1 it is >60 and 29 minutes, respectively. T99.99 values for polio 1 and echo 1 are also long (>60 minutes) at pH 4.5, but HAV data for this pH are not yet available. At pH 9.5, both HAV and echo 1 are inactivated rapidly, with T99.99 values of <0.33 and <0.96 minutes, respectively. Data for polio 1 inactivation at the same condition are not available due to virus assay problems.

Data on iodine levels in test samples (see Figures 7 to 9) indicate that the initial target concentration of >7.5 mg/l was met in all experiments. However, iodine concentrations declined during the 60 minute contact time, with 60 minute residuals of 3.9 to 7.7 mg/l. Some of this loss of iodine is due to demand from the virus preparation because losses were not as great in iodine control samples held at the same temperature for 60 minutes.

The results obtained so far indicate that HAV is rapidly inactivated by one tablet of iodine per quart of buffered HDFW. In contrast, polio 1 and echo 1 are not rapidly inactivated by this concentration of iodine at pH 4.5 or 7.0. At pH 9.0, however, at least echo 1 is rapidly inactivated by this concentration of iodine. These experiments must be repeated to verify the results and to obtain additional data for each virus at each test condition being studied. In addition, data must be obtained for experiments at 25°C.

The finding so far that HAV is less resistant or similar in resistance to iodine inactivation than are the other two test viruses is generally consistent with the results obtained so far using free chlorine. However, some differences can be noted between chlorine and iodine. At pH 9.5, both HAV and echo 1 are rapidly inactivated by one tablet of iodine per quart. In contrast, HAV is inactivated relatively rapidly at pH 9.5 by 1 mg/l free chlorine (T99.99 = 10 minutes), but polio 1 and echo 1 are inactivated quite slowly (T99.99 = 65 and 147 minutes, respectively). At pH 7.0, 1 mg/l free chlorine rapidly inactivates all three viruses (T99.99 <11 minutes), but one tablet of iodine per quart rapidly inactivates only HAV. These

comparisons must be made and interpreted with caution because the results of the iodine experiments are incomplete and because the chlorine concentration of only 1 mg/l is lower on both a weight and molar basis than the iodine concentration of about 8 mg/l. The relative ability of each disinfectant to inactivate these three viruses will become clearer when the results of experiments for their equimolar concentrations (3.6 mg/l iodine versus 1 mg/l chlorine) are available. Virus inactivation rates for 3.6 mg/l iodine are expected to be somewhat slower than those found at one tablet of iodine per quart (about 8 mg/l). Therefore, for those conditions under which virus inactivation is slower by one tablet of iodine per quart than by 1 mg/l chlorine, the lower virucidal activity of iodine should be even more obvious when it is tested at 3.6 mg/l.

V. SUMMARY AND CONCLUSIONS

An important conclusion that can be drawn so far from the results of the disinfection experiments using a dose of 1 mg/l free chlorine in buffered HDFW at pH 4.5, 7.0 and 9.5 and at 5 and 25°C is that the inactivation of the HM175 strain of HAV is greater than or similar to the inactivation of polio 1 and echo 1 under nearly all conditions tested. These results are generally consistent with those of Grabow et al. (1983), who reported that HAV was relatively sensitive to free chlorine, and they are in contrast to the results of Peterson et al. (1983), which suggested that HAV was relatively resistant to free chlorine.

The greater or equal inactivation of HAV compared to polio 1 and echo 1 was also observed using a dose of one tablet of iodine per quart of buffered HDFW at pH 5°C. Based on the results obtaind so far, it appears that HAV is relatively sensitive to inactivation by free chlorine and Army iodine. However, further experiments to be done in waters having considerably greater halogen demand than the waters used in the experiments reported above are needed before definitive conclusions can be drawn about the relative resistance or sensitivity of HAV to different forms of chlorine or iodine in water. As previously noted, the few studies on HAV disinfection reported in the literature suggest that HAV may be quite resistant to combined forms of chlorine. In addition, further chlorine and iodine disinfection studies must be done with other strains of HAV in order to determine if their response is similar to or different from that of the HM175 strain of HAV.

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TABLE 1

Time (Minutes) for 99.99% Inactivation of HAV, Polio 1 & Echo 1 by 1 mg/l Free Chlorine in Buffered Halogen Demand-free Water at pH 4.5, 7.0 and 9.5, and 5°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
HAV	9.9	1.0	10.0
Polio 1	12.0	2.6	65.0
Echo 1	12.5	11.0	147.0

*Times for 99.99% inactivation estimated from graphs of $\log_{10}$ virus survival versus contact time; based on average results of duplicate experiments.

TABLE 2

Time (Minutes) for 99.99% Inactivation of HAV, Polio 1 & Echo 1 by 1 mg/l Free Chlorine in Buffered Halogen Demand-free Water at pH 4.5 and 7.0, and 25°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5**	pH 7.0
HAV	15.0	0.4
Polio 1	0.8	0.6
Echo 1	13.0	1.2

*Times for 99.99% inactivation estimated from graphs of $\log_{10}$ virus survival versus contact time; based on average results of duplicate experiments at pH 7.0.
**Results of only one questionable experiment; see text for details.

TABLE 3

Time (Minutes) for 99.99% Inactivation of HAV, Polio 1 and Echo 1 by about 8 mg/l Iodine in Buffered Halogen Demand-free Water at pH 4.5, 7.0 and 9.5, and 5°C*

Virus	Time (Minutes) for 99.99% Inactivation at: pH 4.5	pH 7.0	pH 9.5
HAV	no data	<1.0	<0.33
Polio 1	>60	>60	no data
Echo 1	>60	29	<0.96

*Times for 99.99% inactivation estimated from graphs of $\log_{10}$ virus survival versus contact time; based on results of only single experiments for each condition.

FIGURE 2. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L CL_2, pH 4.5, 5°C

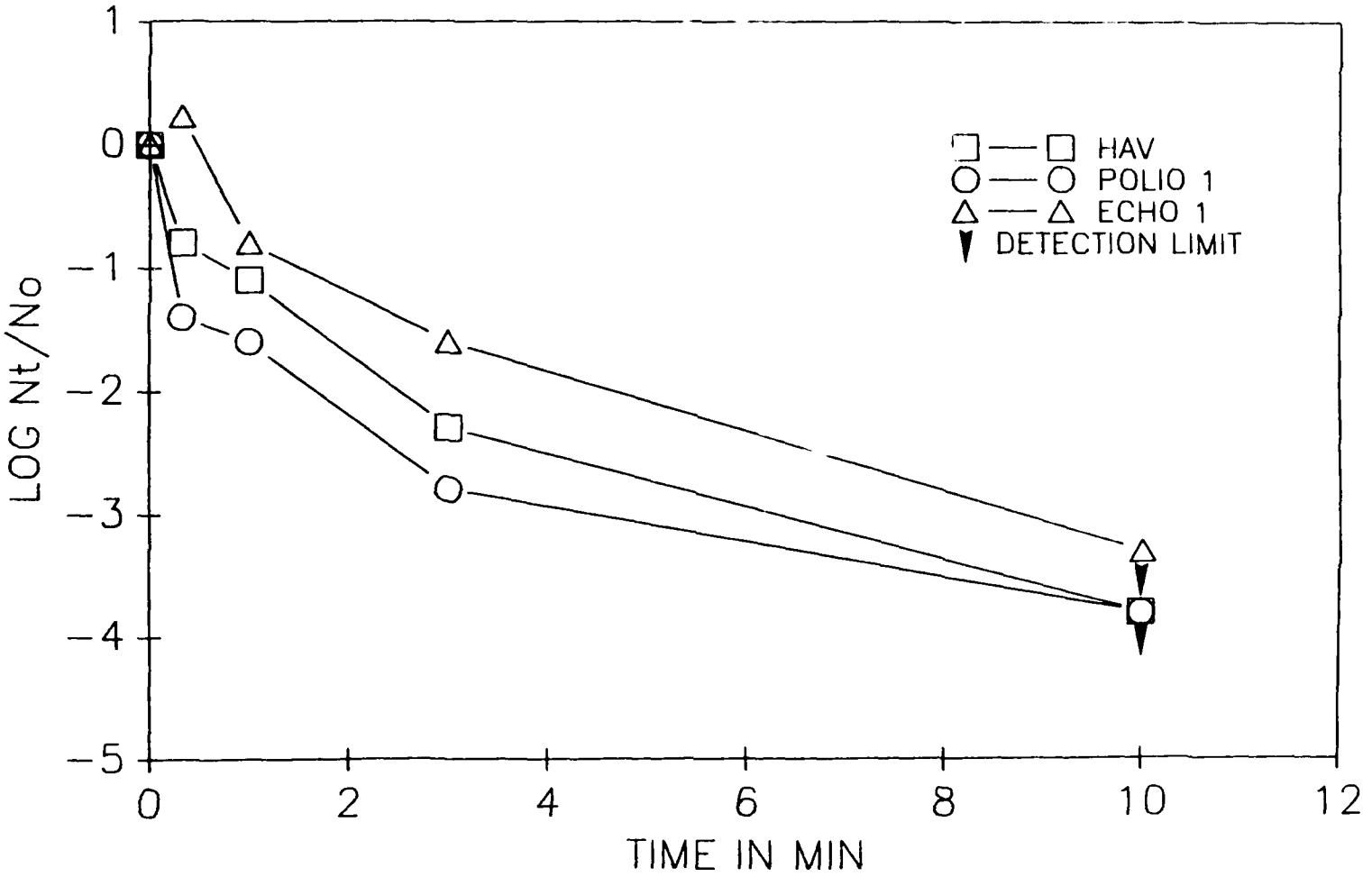

FIGURE 3. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L CL_2, pH 7.0, 5°C

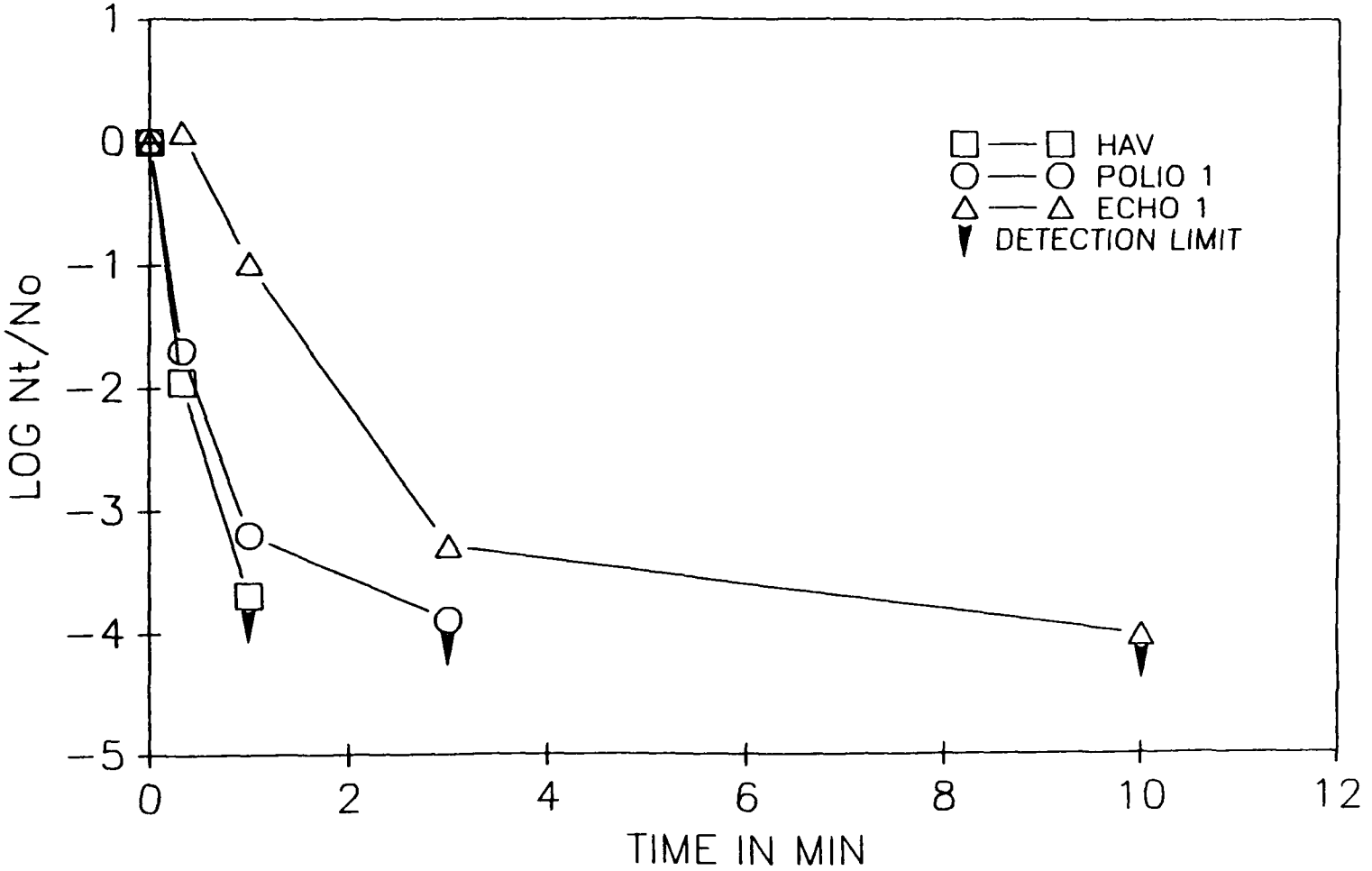

FIGURE 4. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L Cl_2, pH 9.5, 5°C

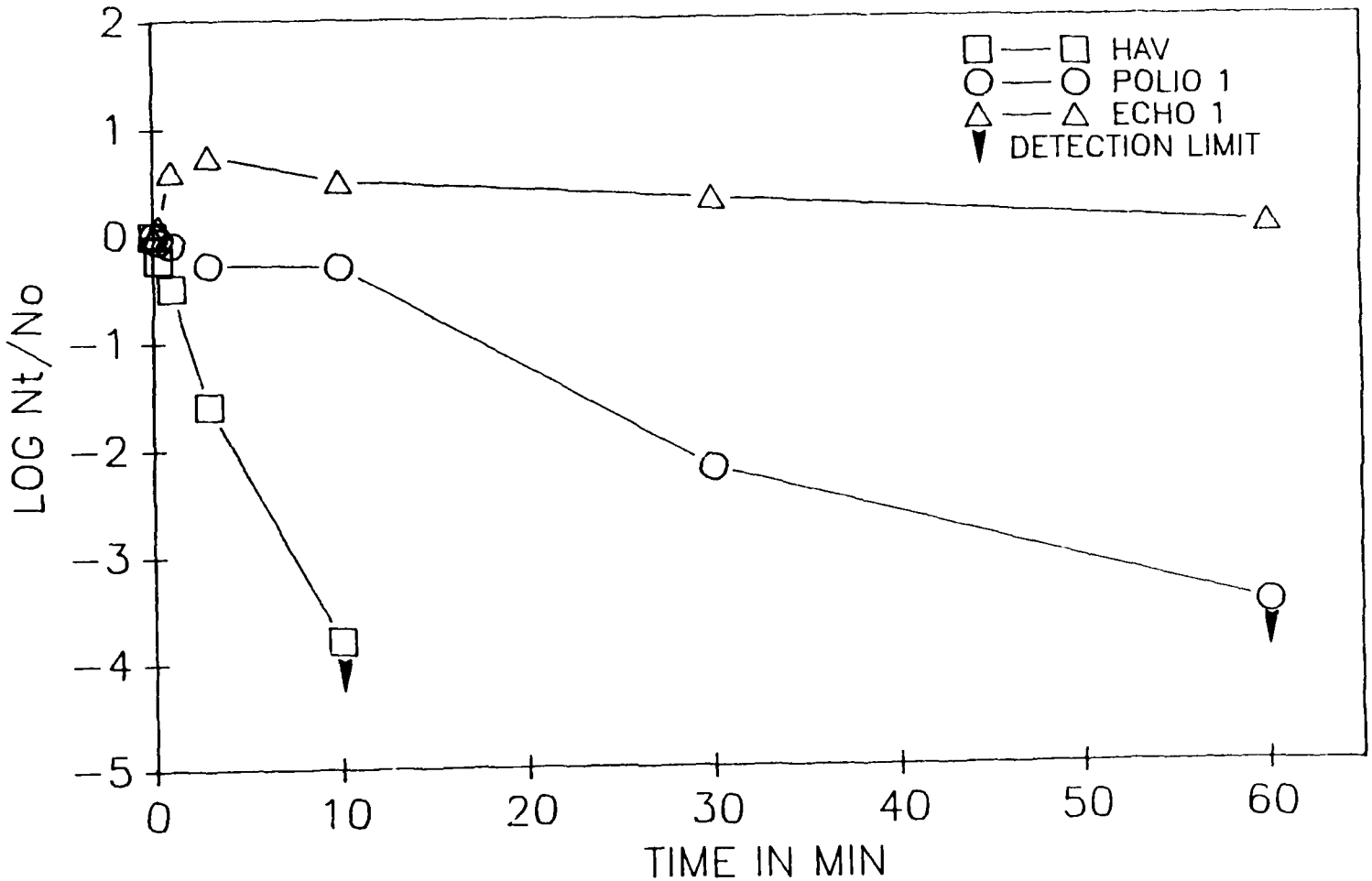

FIGURE 5. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L CL_2, pH 7.0, 25°C

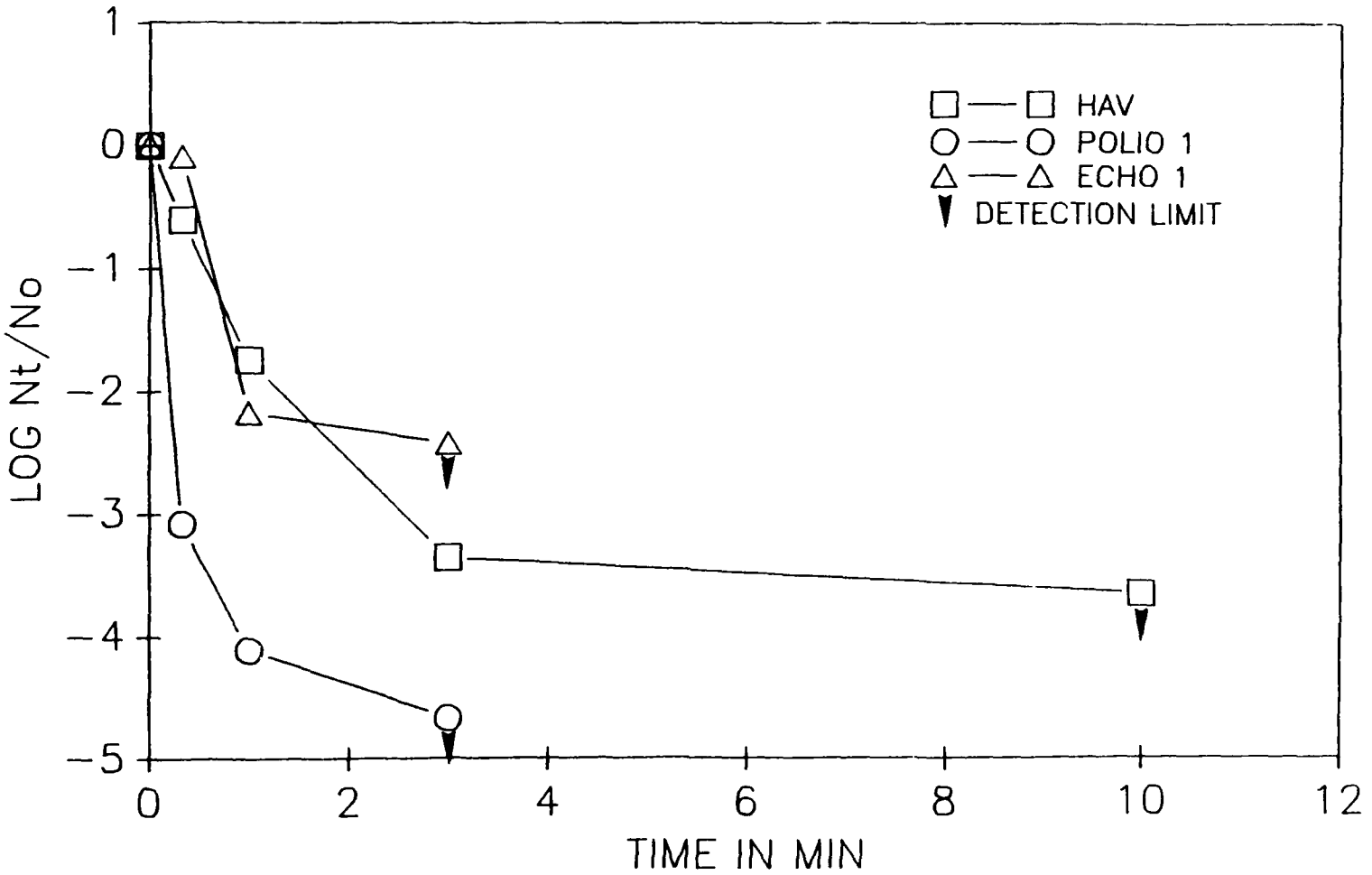

FIGURE 6. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY FREE CHLORINE IN HDF WATER, 1 MG/L CL_2, pH 7.0, 25°C

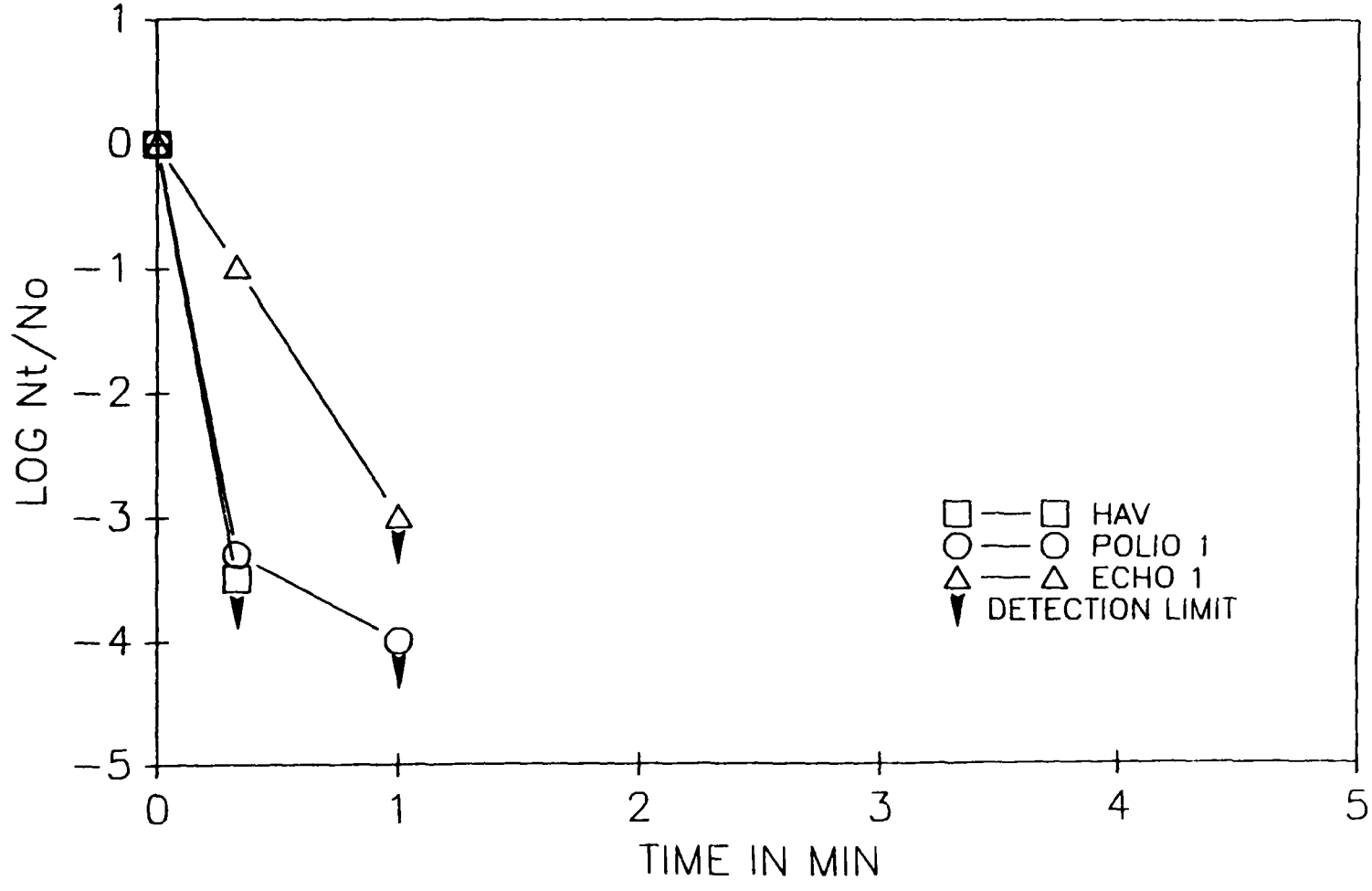

FIGURE 7. INACTIVATION OF POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 4.5, 5°C

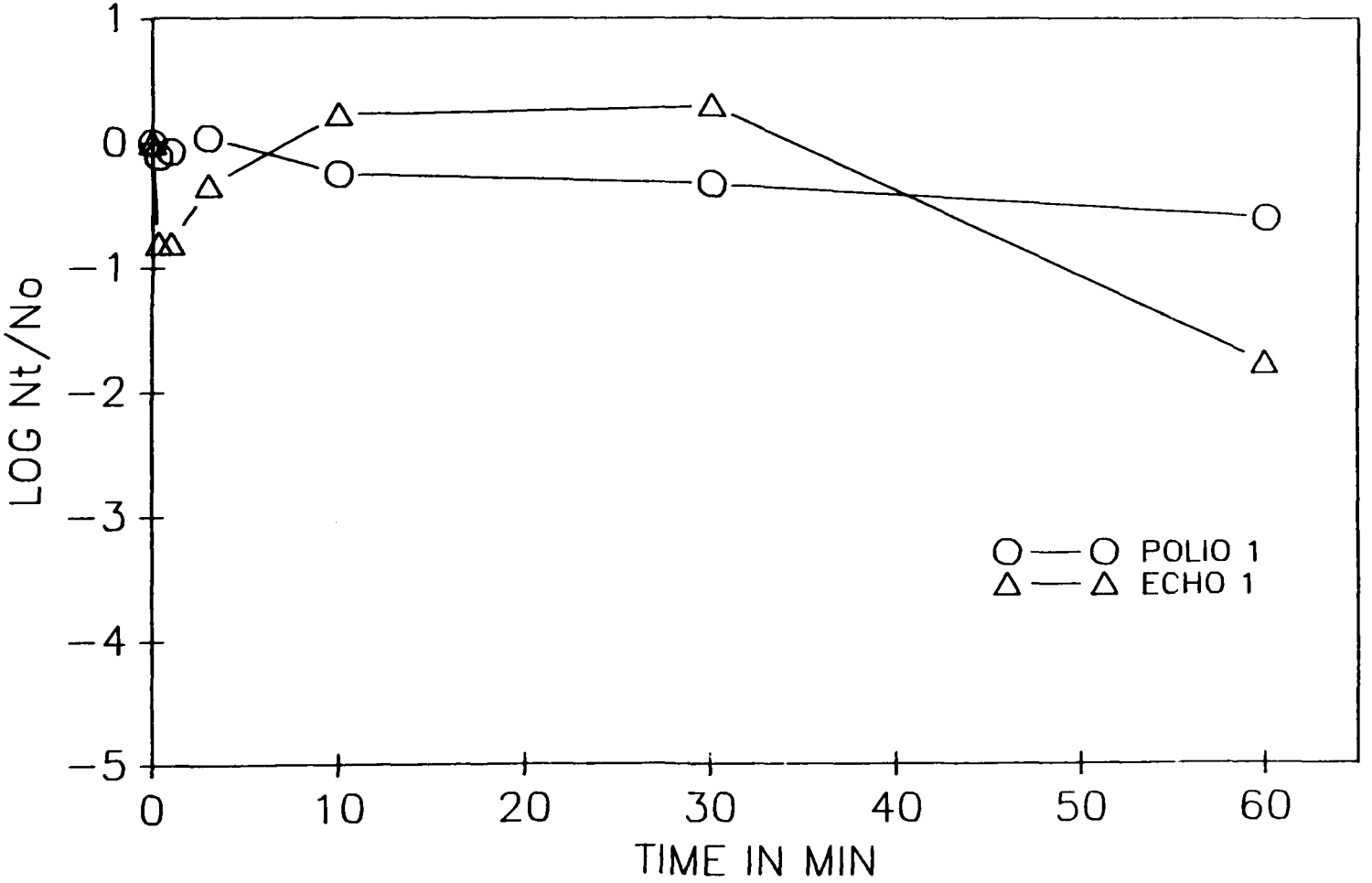

FIGURE 8. INACTIVATION OF HAV, POLIO 1 AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 7.0, 5°C

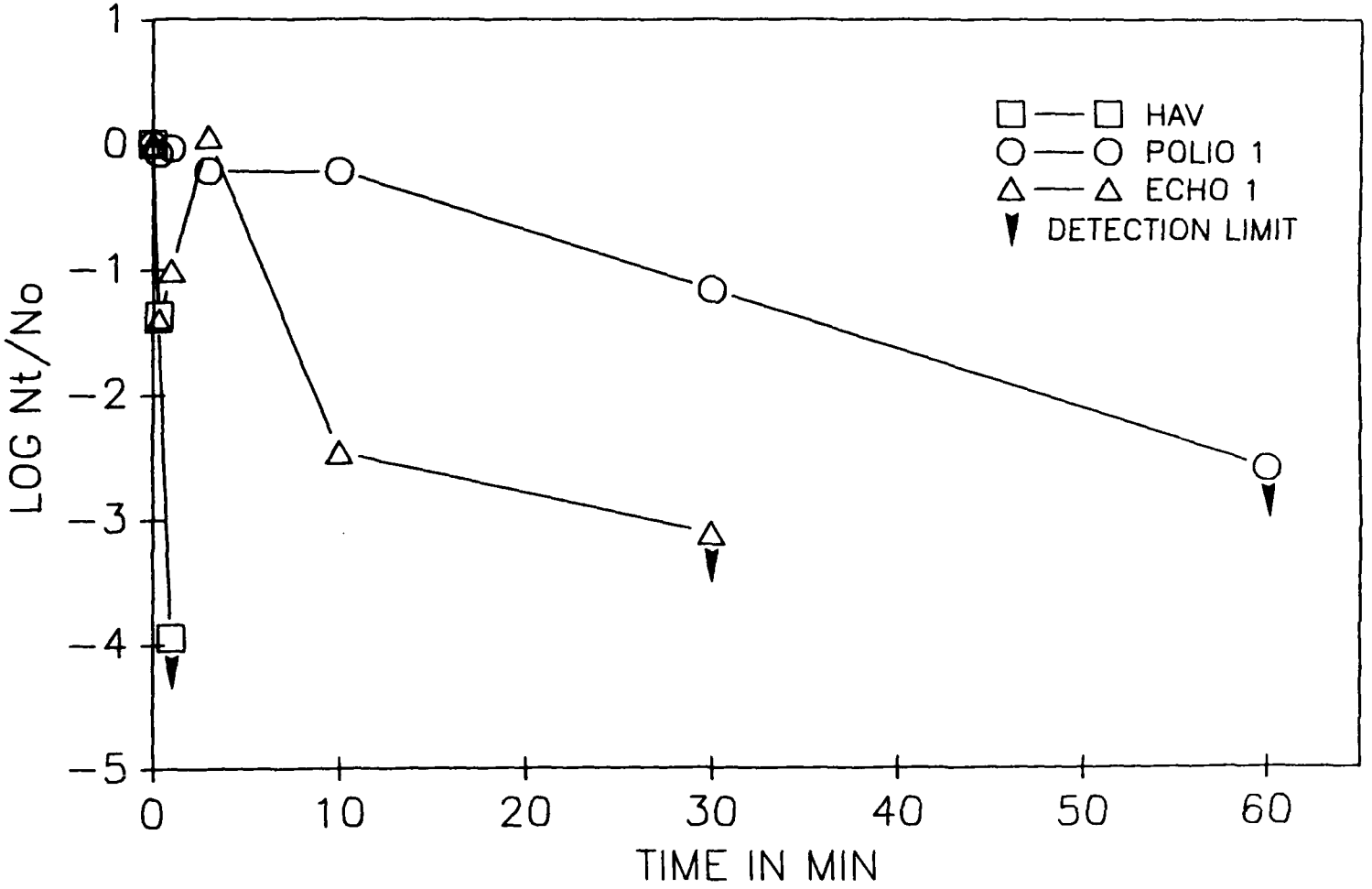

FIGURE 9. INACTIVATION OF HAV AND ECHO 1 BY IODINE IN HDF WATER, 1 TABLET/QUART, pH 9.5, 5°C

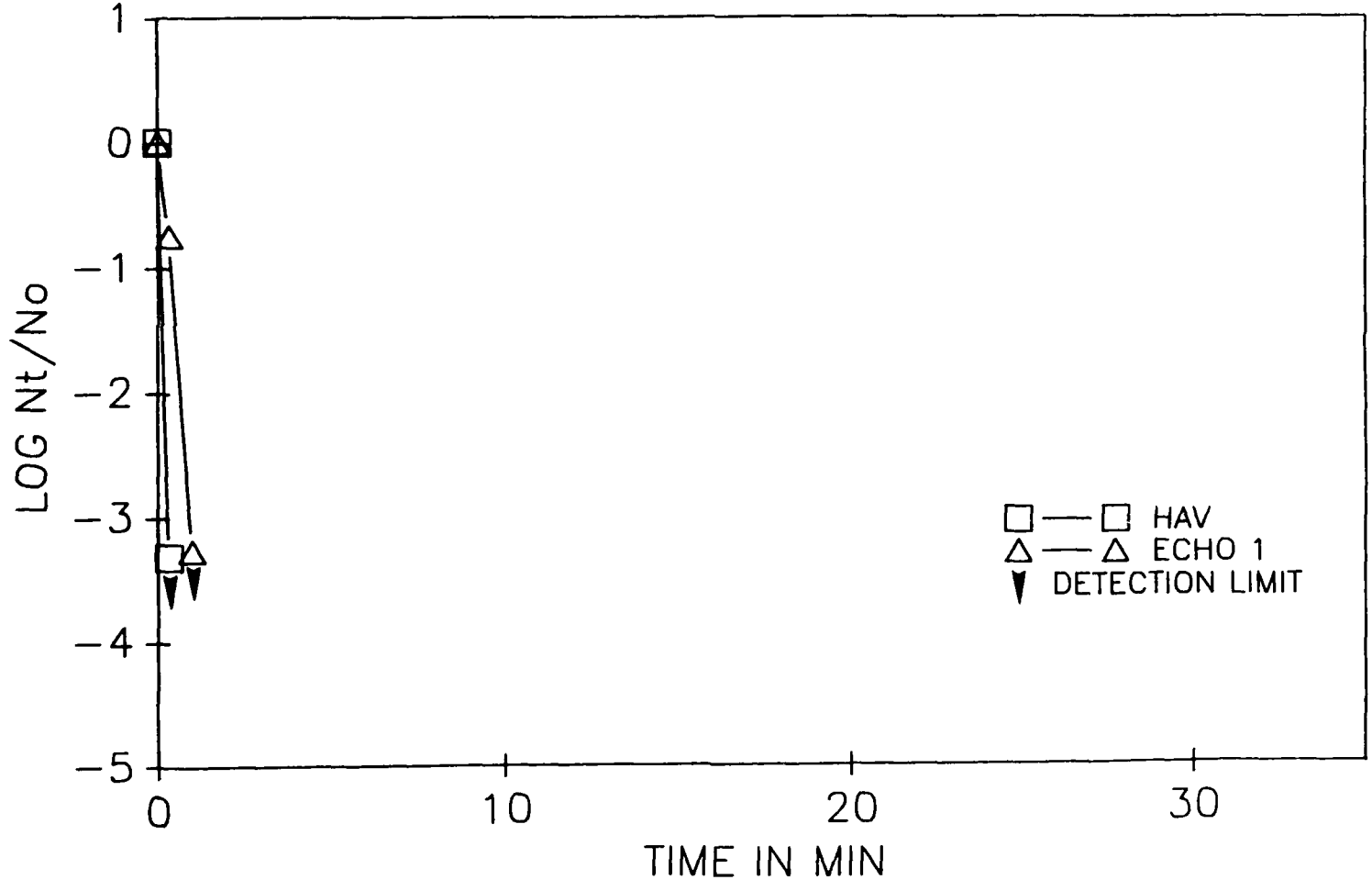

TABLE A1

Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 4.5 and 5°C

Sample and Time (Min.)	Infectious Units/ml Hepatitis A	Poliovirus	Echovirus
		Experiment 1	
Test Sample			
0.33	2.8×10^3	1.68×10^3	1.78×10^4
1.0	4.67×10^3	8.4×10^2	2.71×10^3
3.0	4.67×10^2	<9.3*	8.4×10^1
10	<9.3**	<9.3	<9.3
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	8.4×10^4	3.74×10^4	1.77×10^4
60	2.8×10^4	5.51×10^4	4.11×10^4
		Experiment 2	
Test Sample			
0.33	9.33×10^3	1.12×10^3	2.24×10^3
1.0	2.8×10^3	6.53×10^2	1.77×10^2
3.0	1.02×10^2	6.53×10^1	3.73×10^2
10	<9.3	<9.3	<9.3
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	4.11×10^4	2.6×10^4	7.47×10^4
60	2.99×10^4	1.68×10^4	8.4×10^3
		Means of Experiments 1 and 2	
Test Sample			
0.33	6.10×10^3	1.4×10^3	2.00×10^3
1.0	3.7×10^3	7.47×10^2	2.24×10^3
3.0	2.85×10^2	3.27×10^1	2.29×10^2
10	<4.65	<4.65	<4.65
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	6.26×10^4	3.17×10^4	1.26×10^4
60	2.9×10^4	3.60×10^4	2.48×10^4

*Detection limit for viruses based on 0 (<1) PFU/1.07 ml at 1:10 dilution of reaction mixture.

**ND = no viruses detected at this dilution.

TABLE A2

Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 7.0 and 5°C*

Sample and Time (Min.)	Infectious Units/ml Hepatitis A	Poliovirus	Echovirus
		Experiment 1	
Test Sample			
0.33	1.31×10^2	4.76×10^2	2.24×10^4
1.0	<9.3	2.8×10^1	5.6×10^2
3.0	ND	<9.3	2.8×10^1
10	ND	ND	<9.3
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	2.71×10^4	9.33×10^4	3.64×10^4
60	2.43×10^4	5.23×10^4	3.45×10^4
		Experiment 2	
Test Sample			
0.33	9.34×10^2	1.77×10^3	9.33×10^4
1.0	<9.3	3.72×10^1	7.46×10^3
3.0	ND	<9.3	<9.3
10	ND	ND	ND
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	6.53×10^4	4.57×10^4	3.73×10^4
60	4.67×10^4	3.73×10^4	5.23×10^4
		Means of Experiments 1 and 2	
Test Sample			
0.33	5.33×10^2	1.12×10^3	5.79×10^4
1.0	<4.65	3.26×10^1	4.01×10^3
3.0	ND	<4.65	1.4×10^1
10	ND	ND	<4.65
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	4.62×10^4	6.95×10^4	3.69×10^4
60	3.55×10^4	4.48×10^4	4.36×10^4

*See footnote to Table A1.

TABLE A3

Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 9.5 and 5°C*

Sample and Time (Min.)	Infectious Units/ml Hepatitis A	Poliovirus	Echovirus
		Experiment 1	
Test Sample			
0.33	1.21×10^4	1.68×10^4	1.12×10^5
1.0	1.87×10^3	1.96×10^4	1.12×10^5
3.0	3.73×10^2	1.03×10^3	1.87×10^5
10	<9.3	1.59×10^1	no data
30	ND	3.73×10^1	1.86×10^4
60	ND	<9.3	1.12×10^4
Virus Control			
0	3.73×10^4	2.05×10^4	7.47×10^4
60	5.98×10^4	2.15×10^4	5.6×10^4
		Experiment 2	
Test Sample			
0.33	2.89×10^4	2.33×10^4	1.87×10^4
1.0	1.87×10^4	1.59×10^4	1.4×10^5
3.0	1.21×10^3	1.31×10^4	2.15×10^5
10	<9.3	5.6×10^3	1.6×10^5
30	ND	2.61×10^2	1.03×10^5
60	ND	<9.3	5.6×10^4
Virus Control			
0	3.64×10^4	1.96×10^4	9.33×10^4
60	2.61×10^4	2.8×10^4	4.30×10^4
		Means of Experiments 1 and 2	
Test Sample			
0.33	2.05×10^4	2.0×10^4	6.54×10^4
1.0	1.03×10^4	1.78×10^4	1.26×10^5
3.0	7.92×10^2	1.17×10^4	2.19×10^5
10	<4.65	3.6×10^3	1.6×10^5
30	ND	1.49×10^2	6.08×10^4
60	ND	<4.65	3.36×10^4
Virus Control			
0	3.69×10^4	2.0×10^4	4.20×10^4
60	4.30×10^4	2.48×10^4	4.95×10^4

*See footnote to Table A1.

TABLE A4

Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 4.5 and 25°C*

Sample and Time (Min.)	Infectious Units/ml Hepatitis A	Poliovirus	Echovirus
		Experiment 1	
Test Sample			
0.33	$1.02 \times 10^{4}_{2}$	$3.64 \times 10^{2}_{1}$	$2.04 \times 10^{3}_{1}$
1.0	$7.47 \times 10^{2}_{1}$	3.36×10^{1}	1.68×10^{1}
3.0	1.87×10^{1}	<9.3	<9.3
10	<9.3	ND	ND
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	5.5×10^{4}	4.2×10^{5}	3.64×10^{3}
60	2.8×10^{4}	4.06×10^{5}	1.4×10^{3}

*See footnote to Table A1; Results of only a single experiment available so far.

TABLE A5

Inactivation of HAV, Polio 1 and Echo 1 by 1 mg/l Free Chlorine at pH 7.0 and 25°C*

Sample and Time (Min.)	Infectious Units/ml Hepatitis A	Poliovirus	Echovirus
		Experiment 1	
Test Sample			
0.33	<9.33	2.8×10^1	2.8×10^2
1.0	ND	<9.3	<9.3
3.0	ND	ND	ND
10	ND	ND	ND
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	3.64×10^4	5.6×10^4	2.34×10^4
60	4.67×10^4	1.59×10^4	4.2×10^5
		Experiment 2	
Test Sample			
0.33	<9.3	1.68×10^2	7.0×10^2
1.0	ND	<9.3	<9.3
3.0	ND	ND	ND
10	ND	ND	ND
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	1.49×10^4	5.46×10^5	3.36×10^3
60	1.12×10^4	5.49×10^5	3.64×10^3
		Means of Experiments 1 and 2	
Test Sample			
0.33	<4.65	9.8×10^1	4.9×10^2
1.0	ND	<4.65	<4.65
3.0	ND	ND	ND
10	ND	ND	ND
30	ND	ND	ND
60	ND	ND	ND
Virus Control			
0	2.57×10^4	3.05×10^5	1.34×10^4
60	2.90×10^4	2.82×10^5	2.11×10^5

*See footnote to Table A1.

TABLE A6

Inactivation of HAV, Polio 1 and Echo 1 by 1 Tablet/Quart Iodine at pH 4.5 and 5°C*

Sample and Time (Min.)	Infectious Units per ml Hepatitis A	Poliovirus	Echovirus
Test Sample			
0.33	no data	5.1×10^3	2.0×10^3
1.0	"	5.7×10^3	2.0×10^3
3.0	"	7.3×10^3	6.0×10^3
10	"	3.7×10^3	2.2×10^4
30	"	3.1×10^3	2.6×10^4
60	"	1.7×10^3	2.2×10^2
Virus Control			
0	"	6.7×10^3	1.3×10^4
60	"	6.1×10^3	2.4×10^4

*See footnote to Table A1.

TABLE A7

Inactivation of HAV, Polio 1 and Echo 1 by 1 Tablet/Quart Iodine at pH 7.0 and 5°C*

Sample and Time (Min.)	Infectious Units per ml Hepatitis A	Poliovirus**	Echovirus
Test Sample			
0.33	2.5×10^3	2.2×10^3	3.4×10^2
1.0	<9.3	2.4×10^3	8.6×10^2
3.0	ND	1.6×10^3	1.0×10^4
10	ND	1.6×10^3	3.0×10^1
30	ND	1.8×10^2	<9.3
60	ND	<9.3	ND
Virus Control			
0	5.9×10^4	2.6×10^3	8.8×10^3
60	2.4×10^4	1.4×10^3	1.2×10^4

*See footnote to Table A1.
**Results from poor quality plaque assay.

TABLE A8

Inactivation of HAV, Polio 1 and Echo 1 by 1 Tablet/Quart Iodine at pH 9.5 and 5°C*

Sample and Time (Min.)	Infectious Units per ml Hepatitis A	Poliovirus	Echovirus
Test Sample			
0.33	<9.3	no data	2.2×10^3
1.0	ND	no data	<9.3
3.0	ND	no data	ND
10	ND	no data	ND
30	ND	no data	ND
60	ND	no data	ND
Virus Control			
0	1.4×10^4	no data	1.24×10^4
60	1.6×10^4	no data	1.17×10^4

*See footnote to Table A1.

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ADA 199503

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: University of North Carolina-Chapel Hill; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): CB 7400 Rosenau, Chapel Hill, NC 27599	7b. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701-5010
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-86-C-6053
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO. 3E16277-	TASK NO.	WORK UNIT ACCESSION NO.
62777A	7A878	CA	293

11. TITLE (Include Security Classification)

(U) Inactivation of Hepatitis A Virus (HAV) by Chlorine & Iodine in Water

12. PERSONAL AUTHOR(S)

Mark D. Sobsey

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 10/15/85 TO 10/14/86	November 1986	41

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	10	
06	09	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Hepatitis A Virus, Water, Disinfection, Chlorine, Iodine, Inactivation

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Batch laboratory studies were done on the inactivation of HAV, poliovirus 1 and echovirus 1 by 1 mg/l free chlorine and ca. 8 mg/l Army iodine in halogen demand-free water at pH 4.5, 7.0 and 9.5 and 5 and 25°C. HAV was rapidly inactivated by both halogens under the conditions tested so far, with times for 99.99% inactivation of less than 15 minutes. In contrast, polio 1 and echo 1 were not rapidly inactivated under some conditions, notably pH 9.5 for chlorine and pH 4.5 and 7.0 for iodine. Further studies are in progress to determine the sensitivity of these viruses to chlorine and iodine in the presence of appreciable halogen demand and other potential interferences.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
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DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE